

Chronic Ingestion of Mn3O4 by Rats
Tissue Accumulation and Distribution of
Manganese in Two Generations

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**CHRONIC INGESTION OF Mn_3O_4 BY RATS:
TISSUE ACCUMULATION AND DISTRIBUTION
OF MANGANESE IN TWO GENERATIONS**

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Sprague-Dawley rats were chronically exposed to particulate Mn_3O_4 through two generations. At specific ages, observations were made of growth, tissue content, and distribution of Mn and Fe as affected by chronic exposure to Mn through an Fe-sufficient diet and an Fe-deficient diet. Chronic dietary Mn_3O_4 resulted in dose-related increases in Mn accumulation, and a concomitant Fe deficiency promoted Mn accumulation. In general, the addition of substantial amounts of Mn to either diet depressed tissue Fe levels.

INTRODUCTION

Large overdoses of Mn are known to increase concentrations of Mn in tissues and decrease liver Fe (Jarvinen and Ahlström, 1975; Britton and Cotzias, 1966; Cotzias et al., 1976). However, the symptoms of Mn overdose appear to be mediated through interference in the metabolism of Fe. There is a competitive relation between Fe and Mn for the intestinal transport mechanism. Fe has a greater inhibitory effect on Mn absorption than Mn has on Fe absorption (Pollack et al., 1965). The body Fe state must also be taken into account, as an initial Fe deficiency increases Fe absorption, which in turn may affect Mn absorption (Howard and Jacobs, 1972; Thomson and Valberg, 1972).

Populations susceptible to increased intestinal absorption of Mn include the very young and the Fe-deficient. The preweanling rat has higher absorption, higher retention, slower turnover, and significant deposition of Mn in the liver, kidney, and brain (Cahill et al., 1980). Individuals with increased Fe absorption (the Fe-deficient) have increased Mn absorption as well. However, Mn absorption is enhanced in Fe deficiency, primarily due to increased uptake. The adult animal tends to compensate for increased Mn absorption by increased excretion of Mn in the feces (Thomson et al., 1971).

Differences in Mn absorption between the newborn and the adult may reflect differences in gastrointestinal function, such as the capacity to

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absorb molecules of a certain size and quality and the quantity of digestive enzymes. It has also been suggested that the newborn rat has reduced hepatic excretory function, which decreases the ability to excrete compounds into the bile (Klaasen, 1973).

In this study, rats were chronically exposed to particulate Mn_3O_4 from d 2 of gestation of the F_1 generation through 24 d of age in the F_2 generation. Observations were made of growth, tissue content, and distribution of Mn and Fe as affected by chronic exposure to Mn_3O_4 through a normal Fe diet and a low-Fe diet. These evaluations were made at selected periods and included pregnant and lactating dams (F_1 dams), weanlings (F_1 and F_2), adolescents (F_1), adults (F_1), and neonates (F_2).

MATERIALS AND METHODS

A total of 255 time-pregnant Long Evans rats (Blue Spruce Farms, Altamont, N.Y.) were shipped to our laboratory on d 1 of gestation. They were housed in individual cages with a 12-12 light-dark cycle and had access to feed and tap water *ad libitum*. Treatment began on d 2 of gestation and was continued throughout the experiment.

On arrival at the laboratory, the rats were divided into two groups: one group to be maintained on a low-Fe diet (20 $\mu\text{g/g}$), the second to be maintained on a normal Fe diet (240 $\mu\text{g/g}$). Each group was then divided into four subgroups to receive different amounts of Mn in the diet. Before the addition of Mn_3O_4 , the basal casein diet¹ contained 50 $\mu\text{g/g}$ Mn as manganese sulfate. Particulate Mn_3O_4 was then added to both diets to achieve a total Mn concentration of 400, 1100, or 3550 $\mu\text{g/g}$. These experimental diets were prepared in 10-kg amounts and stored at 10°C. Each shipment of these diets was analyzed for Fe content by the WARF Institute, Madison, Wisc. Mn and Fe concentrations in the drinking water were 0.05 ± 0.02 ppm and <0.5 ppm, respectively, as determined by atomic absorption spectrophotometry.

Mn_3O_4 was obtained from Alpha Products, Danvers, Maine. Purity was determined to be 98% by atomic absorption spectrophotometry. Particle size and distribution were determined by light microscopy (Grainger Laboratories, Inc., Raleigh, N.C.) and are presented in Table 1. A general outline of the study, indicating ages at which animals were sacrificed and tissues taken for analysis, is shown in Fig. 1.

At term, pups were delivered naturally and d 1 postpartum the F_1 litters were normalized to 6 pups (3 males and 3 females), cross-fostering when necessary. Pups were weaned on d 24 after birth. At 92-94 d of age, F_1 females were mated to nonlittermate males of the same dose group. Half of the dams delivered naturally and half were delivered by cesarean section. F_2 pups that were delivered naturally were normalized to 6 pups

¹ Base diets TD-76476 (240 mg/kg Fe) and TD-76475 (20 mg/kg Fe) were obtained from Teklad Test Diets, Madison, Wis.

TABLE 1. Particle size and distribution of Mn_3O_4

Particle size (μm)	Percent
≤ 0.3	3.0
0.31-0.60	19.5
0.61-0.90	13.0
0.91-1.20	20.5
1.21-1.50	9.5
1.51-1.80	12.5
1.81-2.10	5.5
2.11-2.40	7.0
2.41-2.70	1.5
2.71-3.00	3.5
> 3.01	4.5

(3 males and 3 females) per litter. Tissues were removed from the dams and the F_2 neonates. Liver, kidney, and brain (cerebrum) were taken at all sacrifice ages. Testes were taken from males at all sacrifice ages. Blood was collected at 24 and 40 d of age in the F_1 generation. Adrenals, pituitary, and spleen were taken at 224 d of age. Milk was collected from P_0 and F_1 lactating dams 24 d postpartum. Placenta was collected from F_1 term dams.

All organs, after dissection, were weighed, placed in plastic vials, and stored at $-60^\circ C$. Before analysis tissues were thawed, and a portion was removed and analyzed for Mn and Fe content by atomic absorption spectrophotometry (Rehnberg et al., 1980).

Statistical analyses included analysis of variance (ANOVA) and Dunnett's multiple comparison test (Dunnett, 1955).

RESULTS

Rats chronically exposed to Mn_3O_4 in a normal Fe diet showed no change in growth rate. However, males and females on the low-Fe diet supplemented with Mn showed Mn-related depressions in body weight. Body weights of the Fe-deficient males receiving 1100 $\mu g/g$ Mn in the diet were depressed 20-30% through 100 d of age. Body weights of females from the same treatment group were depressed 10-15% through 60 d of age. Body weights of both males and females receiving a 400 $\mu g/g$ Mn, low-Fe diet were depressed 10-15% through 60 d of age. Beyond 100 d of treatment, no body weight deficits were observed in any treatment group.

Mortality of animals maintained on the low-Fe diet supplemented with 3550 $\mu g/g$ Mn was greater than 75% at 40 d of age. A small number of these animals were killed at 40 d of age for tissue samples. All remaining animals in this dose group failed to survive beyond 50 d of age. Blood analyses on these animals indicated severely depressed Fe levels. Their blood Fe values were only 15 and 21% of those of all animals on an Fe-

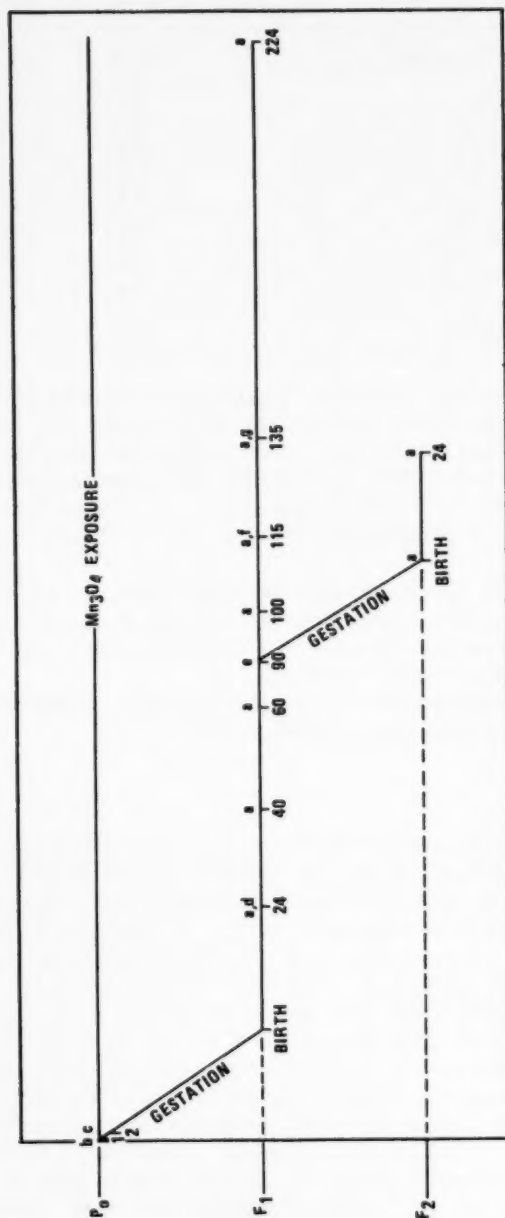


FIGURE 1. Outline of Mn₂O₄ exposure and ages (days) when observations and/or tissues were taken. (a) Tissue taken for Mn and Fe analysis, (b) conception of F₁, (c) Mn₂O₄ treatment began, (d) weaned, (e) conception of F₂, (f) term dams, (g) lactating dams.

sufficient diet and control animals on an Fe-deficient diet. Liver analyses indicated very low Fe concentrations, but not significantly lower than those in the Fe-deficient controls. Mn concentrations in the livers of these animals were almost four times those observed in the Fe-deficient controls and twice those in animals receiving $1100 \mu\text{g/g}$ Mn in the low-Fe diet.

Liver Mn concentrations are presented in Table 2. Liver Mn accumulation in Fe deficiency was significantly increased in term and lactating dams, 40-d-old animals, F_1 and F_2 weanlings, and term pups compared to corresponding animals fed the normal Fe diet. In general, there was a dose-related increase in liver Mn in both Fe groups.

Concentrations of Mn in kidneys of control and experimental animals are presented in Table 3. Mn concentrations in the kidneys of animals fed the Fe-deficient diet are slightly higher than those in the kidneys of animals on the normal Fe diet with the exception of F_2 term pups, term dams, and 224-d-old males and females. Dose-related increases of Mn in the kidney are less obvious than those in the liver.

Animals fed Mn_3O_4 in the low-Fe diet had slightly higher dose-related Mn concentrations in the cerebrum than animals on the normal Fe diet (Table 4). Concentrations of Mn in the cerebrum were highest in the F_1 and F_2 weanlings. Mn concentrations in the cerebrum in the Fe-deficient F_2 weanlings were 50-70% higher than in the Fe-deficient F_1 weanlings receiving the same Mn_3O_4 treatment. The lowest brain Mn concentrations were found in the F_2 neonates. Mn concentrations reported for the pups are for whole brain.

Although Mn concentrations in the pituitary were measured only at 224 d of age, Mn exposure resulted in a 40-80% increase in both Fe status groups over controls ($240 \mu\text{g/g}$ Fe).

Significant dose-related increases in testicular Mn were seen only in 24-d-old F_1 weanlings (Table 5). Testicular Mn concentrations were unaffected by the Mn or Fe regimen at all other measurement periods. Blood Mn concentrations were linearly related to dose at both ages measured and were higher in the Fe-deficient animals. As seen in Table 5, concentrations in milk or placenta were generally unaffected by treatment. Spleen Mn levels were also unaffected by Mn dose. Adrenal concentrations of Mn were generally unaffected by treatment; however, concentrations in the 3550 ppm group were significantly higher than those in controls.

Table 6 shows concentrations of Fe in the liver for rats chronically exposed to Mn_3O_4 in a normal Fe diet or a low-Fe diet. All animals on the low-Fe diet had significantly lower liver Fe concentrations than controls on a normal Fe diet. The addition of substantial amounts of Mn_3O_4 to the low-Fe diet caused further decreases of liver Fe in most cases. Animals fed the normal Fe diet supplemented with Mn_3O_4 tended to have Mn dose-related depressions in liver Fe, with the exception of F_2 term pups.

Fe concentrations were also determined in kidney, cerebrum, adrenals, and testes at selected sacrifice times. Tissues from animals maintained on

TABLE 2. Concentration of Manganese ($\mu\text{g/g}$ wet weight) in Liver during Chronic Mn_3O_4 Exposure

Age ^d	Generation	Sex ^b	Fe = 240 µg/g diet; dietary Mn (µg/g)=			Fe = 20 µg/g diet ^c ; dietary Mn (µg/g) =			
			50	400	1100	3550	50	400	1100
24	F ₁	M+F	(5) ^d 2.1 ± 0.2 ^e (11)	(6) 4.0 ± 0.8 ^f (12)	(6) 6.7 ± 3.6 ^f (12)	(6) 17.0 ± 0.6 ^f (13)	(5) 3.1 ± 0.5 ^f (13)	(6) 4.9 ± 1.2 ^{f,g} (12)	(6) 6.9 ± 1.9 ^{f,g} (12)
40	F ₁	M+F	(6) 2.0 ± 0.2 (6)	(6) 2.6 ± 0.2 ^f (6)	(5) 2.8 ± 0.4 ^f (5)	(6) 4.6 ± 0.6 ^f (6)	(6) 2.5 ± 0.3 ^f (6)	(6) 3.1 ± 0.6 ^{f,g} (6)	(6) 4.6 ± 0.7 ^{f,g} (6)
60	F ₁	M	(5) 2.6 ± 0.5 (5)	(6) 2.6 ± 0.2 (6)	(6) 2.6 ± 0.1 (6)	(6) 3.0 ± 0.3 (6)	(6) 2.7 ± 0.3 (6)	(6) 2.6 ± 0.2 (6)	(5) 3.5 ± 0.5 ^{f,g} (5)
60	F ₁	F	(12) 2.1 ± 0.2 (12)	(12) 2.6 ± 0.3 ^f (12)	(12) 2.5 ± 0.2 ^f (12)	(12) 3.5 ± 0.3 ^f (12)	(12) 2.4 ± 0.2 (12)	(12) 2.4 ± 0.1 ^f (12)	(12) 2.9 ± 0.2 ^{f,g} (12)
100	F ₁	M+F	(8) 2.2 ± 0.2 (8)	(11) 2.7 ± 0.3 ^f (11)	(10) 2.6 ± 0.4 ^f (10)	(10) 3.3 ± 0.5 ^f (10)	(10) 2.5 ± 0.3 ^f (10)	(8) 2.8 ± 0.4 ^{f,g} (8)	(10) 2.9 ± 0.4 ^{f,g} (10)
115	F ₁	F	(8) 2.0 ± 0.4 (8)	(9) 2.5 ± 0.2 ^f (9)	(5) 2.4 ± 0.2 ^f (5)	(5) 3.1 ± 0.3 ^f (5)	(5) 2.8 ± 0.3 ^f (5)	(10) 2.9 ± 0.3 ^f (10)	(8) 3.0 ± 0.2 ^f (8)
135	F ₁	F	(8) 2.8 ± 0.1 (8)	(7) 3.7 ± 0.4 ^f (7)	(8) 3.4 ± 0.2 ^f (8)	(8) 4.7 ± 0.4 ^f (8)	(7) 3.7 ± 0.4 ^f (7)	(8) 3.9 ± 0.3 ^f (8)	(8) 4.5 ± 0.6 ^{f,g} (8)
224	F ₁	M	(5) 2.6 ± 0.3 (5)	(8) 2.6 ± 0.3 (8)	(8) 3.0 ± 0.5 (8)	(8) 3.5 ± 0.5 ^f (8)	(8) 2.5 ± 0.3 (8)	(8) 3.1 ± 0.3 ^g (8)	(8) 3.6 ± 0.5 ^{f,g} (8)
224	F ₁	F	(7) 2.9 ± 0.6 (7)	(10) 3.0 ± 0.5 (10)	(10) 3.4 ± 0.3 (10)	(10) 4.0 ± 0.4 ^f (10)	(10) 2.7 ± 0.3 (10)	(8) 3.4 ± 0.5 ^g (8)	(h) (10)
Term pups	F ₂	M+F	(8) 0.5 ± 0.1 (8)	(10) 0.7 ± 0.2 (10)	(10) 0.8 ± 0.1 ^f (10)	(10) 1.2 ± 0.7 ^f (10)	(10) 0.7 ± 0.1 ^f (10)	(10) 1.0 ± 0.4 ^{f,g} (10)	(10) 1.2 ± 0.3 ^{f,g} (10)
24	F ₂	M+F	(8) 2.9 ± 0.3 (8)	(10) 4.3 ± 0.5 ^f (10)	(10) 5.8 ± 2.1 ^f (10)	(10) 7.7 ± 2.9 ^f (10)	(10) 3.9 ± 0.4 ^f (10)	(10) 5.2 ± 0.8 ^{f,g} (10)	(10) 8.2 ± 3.6 ^{f,g} (10)

^aThe 115-d-old females were full-term pregnant females; the 135-d-old females had delivered and nursed litters for 24 d.^bMale and female values combined because of lack of significant ($p < 0.05$) sex difference at specified age.^cThe dietary Fe content caused a significant ($p < 0.05$) difference in Mn concentration.^dSample number.^eMean $\pm$ SD.^fSignificantly different from 50 (control-240 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).^gSignificantly different from 50 (20 ppm Fe) group by ANOVA and Dunnett's multiple comparison test.^hInsufficient F₁ offspring to complete this group.

TABLE 3. Concentration of Manganese ($\mu\text{g/g}$ wet weight, mean $\pm$ SD) in Kidney during Chronic Mn_2O_3 Exposure

Age ^d	Generation	Sex ^b	Fe = 240 $\mu\text{g/g}$ diet; dietary Mn ($\mu\text{g/g}$) =				Fe = 20 $\mu\text{g/g}$ diet; dietary Mn ($\mu\text{g/g}$) =			
			50	400	1100	3550	50	400	1100	
24	F ₁	M	(5) ^c 0.6 $\pm$ 0.1 ^d (11)	(6) 0.9 $\pm$ 0.2 ^e (12)	(6) 1.5 $\pm$ 0.5 ^e (12)	(6) 2.6 $\pm$ 1.4 ^e (13)	(6) 0.8 $\pm$ 0.1 ^e (13)	(6) 1.4 $\pm$ 0.2 ^{e,f} (12)	(6) 1.2 $\pm$ 0.3 ^{e,f} (12)	
40	F ₁	M+F	(6) 1.3 $\pm$ 0.3 (6)	(6) 1.3 $\pm$ 0.1 (6)	(6) 1.4 $\pm$ 0.2 (6)	(6) 2.0 $\pm$ 0.6 ^e (6)	(6) 1.4 $\pm$ 0.2 (6)	(6) 1.3 $\pm$ 0.2 (6)	(6) 2.0 $\pm$ 0.5 ^{e,f} (6)	
60	F ₁	M	(5) 1.5 $\pm$ 0.6 (5)	(6) 1.4 $\pm$ 0.2 (6)	(6) 1.6 $\pm$ 0.2 (6)	(6) 1.4 $\pm$ 0.1 (6)	(6) 1.5 $\pm$ 0.2 (6)	(6) 1.5 $\pm$ 0.1 (6)	(5) 1.8 $\pm$ 0.1 ^f (5)	
60	F ₁	F	(6) 1.0 $\pm$ 0.1 (6)	(6) 1.1 $\pm$ 0.1 (6)	(6) 1.2 $\pm$ 0.1 (6)	(6) 1.2 $\pm$ 0.2 (6)	(5) 1.2 $\pm$ 0.1 ^e (5)	(6) 1.4 $\pm$ 0.1 ^{e,f} (6)	(6) 1.5 $\pm$ 0.1 ^{e,f} (6)	
100	F ₁	M	(6) 1.2 $\pm$ 0.1 (6)	(6) 1.3 $\pm$ 0.2 (6)	(6) 1.3 $\pm$ 0.1 (6)	(6) 1.6 $\pm$ 0.2 ^e (6)	(5) 1.3 $\pm$ 0.1 (5)	(6) 1.3 $\pm$ 0.2 (6)	(6) 1.5 $\pm$ 0.1 ^{e,f} (6)	
100	F ₁	F	(8) 0.8 $\pm$ 0.1 (8)	(11) 1.1 $\pm$ 0.1 (11)	(10) 1.0 $\pm$ 0.2 (10)	(10) 1.0 $\pm$ 0.1 ^e (10)	(10) 0.9 $\pm$ 0.2 (10)	(8) 1.1 $\pm$ 0.2 ^e (8)	(10) 1.3 $\pm$ 0.4 ^e (10)	
115	F ₁	F	(8) 0.6 $\pm$ 0.1 (8)	(9) 0.7 $\pm$ 0.1 (9)	(5) 0.8 $\pm$ 0.2 (5)	(5) 0.9 $\pm$ 0.1 ^e (5)	(5) 0.6 $\pm$ 0.1 (5)	(10) 0.7 $\pm$ 0.1 (10)	(8) 0.9 $\pm$ 0.1 ^{e,f} (8)	
135	F ₁	F	(8) 0.9 $\pm$ 0.2 (8)	(7) 1.2 $\pm$ 0.2 (7)	(8) 1.2 $\pm$ 0.2 (8)	(8) 1.5 $\pm$ 0.1 ^e (8)	(7) 1.0 $\pm$ 0.1 (7)	(8) 1.4 $\pm$ 0.2 ^{e,f} (8)	(8) 1.4 $\pm$ 0.3 ^{e,f} (8)	
224	F ₁	M	(5) 0.9 $\pm$ 0.2 (5)	(8) 1.0 $\pm$ 0.1 (8)	(8) 0.9 $\pm$ 0.1 (8)	(8) 1.2 $\pm$ 0.2 (8)	(8) 0.9 $\pm$ 0.2 (8)	(8) 1.1 $\pm$ 0.1 (8)	(8) 0.9 $\pm$ 0.2 (8)	
224	F ₁	F	(7) 1.0 $\pm$ 0.2 (7)	(11) 1.2 $\pm$ 0.1 (11)	(10) 1.2 $\pm$ 0.2 (10)	(10) 1.2 $\pm$ 0.3 ^e (10)	(10) 1.0 $\pm$ 0.2 (10)	(8) 1.2 $\pm$ 0.1 (8)	(10) 1.2 $\pm$ 0.1 (10)	
Term pups	F ₂	M+F	(8) 0.3 $\pm$ 0.1 (8)	(10) 0.3 $\pm$ 0.1 (10)	(10) 0.4 $\pm$ 0.1 (10)	(10) 0.4 $\pm$ 0.1 (10)	(10) 0.4 $\pm$ 0.1 (10)	(10) 0.4 $\pm$ 0.1 (10)	(10) 0.4 $\pm$ 0.1 (10)	
24	F ₂	M+F	(8) 0.8 $\pm$ 0.1 (8)	(10) 0.9 $\pm$ 0.1 (10)	(10) 1.3 $\pm$ 0.4 ^e (10)	(10) 1.7 $\pm$ 0.5 ^e (10)	(10) 0.8 $\pm$ 0.3 (10)	(10) 1.4 $\pm$ 0.2 ^{e,f} (10)	(10) 2.6 $\pm$ 1.8 ^{e,f} (10)	

^dThe 115-d-old females were full-term pregnant females; the 135-d-old females had delivered and nursed litters for 24 d.

^bMale and female values combined because of a lack of significant ($p < 0.05$) sex difference at specified age.

^cSample number.

^dMean $\pm$ SD.

^eSignificantly different from 50 (control-240 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).

^fSignificantly different from 50 (20 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).

^gInsufficient F₁ offspring to complete this group.

TABLE 4. Concentration of Manganese ($\mu\text{g/g}$ wet weight, mean $\pm$ SD) in Cerebrum during Chronic Mn_2O_3 Exposure

Age ^a	Generation	Sex ^b	Fe = 240 $\mu\text{g/g}$ diet; dietary Mn ($\mu\text{g/g}$) =				Fe = 20 $\mu\text{g/g}$ diet ^c ; dietary Mn ($\mu\text{g/g}$) =			
			50	400	1100	3550	50	400	1100	
24	F1	M	(5) ^c 0.5 $\pm$ 0.1 ^d	(6) 0.8 $\pm$ 0.1 ^e	(6) 1.4 $\pm$ 0.5 ^e	(6) 2.7 $\pm$ 0.6 ^e	(6) 0.6 $\pm$ 0.2	(6) 1.1 $\pm$ 0.2 ^{e,f}	(6) 1.5 $\pm$ 0.4 ^{e,f}	
40	F1	M+F	(11) 0.5 $\pm$ 0.1	(12) 0.6 $\pm$ 0.1	(12) 0.7 $\pm$ 0.1 ^e	(13) 1.1 $\pm$ 0.3 ^e	(12) 0.6 $\pm$ 0.1	(12) 0.7 $\pm$ 0.1 ^{e,f}	(12) 0.9 $\pm$ 0.2 ^{e,f}	
60	F1	M+F	(11) 0.4 $\pm$ 0.1	(12) 0.5 $\pm$ 0.1	(12) 0.6 $\pm$ 0.1 ^e	(12) 0.7 $\pm$ 0.1 ^e	(12) 0.5 $\pm$ 0.1	(12) 0.6 $\pm$ 0.1 ^{e,f}	(11) 0.7 $\pm$ 0.1 ^{e,f}	
100	F1	M	(6) 0.4 $\pm$ 0.04	(6) 0.5 $\pm$ 0.04 ^e	(6) 0.5 $\pm$ 0.02 ^e	(6) 0.6 $\pm$ 0.05 ^e	(6) 0.4 $\pm$ 0.02	(6) 0.5 $\pm$ 0.02 ^{e,f}	(6) 0.5 $\pm$ 0.03 ^{e,f}	
100	F1	F	(6) 0.4 $\pm$ 0.4	(6) 0.4 $\pm$ 0.3	(6) 0.4 $\pm$ 0.3	(6) 0.5 $\pm$ 0.1	(6) 0.4 $\pm$ 0.04	(6) 0.4 $\pm$ 0.03	(6) 0.5 $\pm$ 0.03	
224	F1	M+F	(13) 0.4 $\pm$ 0.1	(15) 0.4 $\pm$ 0.1	(16) 0.4 $\pm$ 0.1	(16) 0.5 $\pm$ 0.1 ^e	(15) 0.4 $\pm$ 0.1	(16) 0.4 $\pm$ 0.1	(8) 0.5 $\pm$ 0.1	
Term pups ^g	F2	M+F	(7) 0.3 $\pm$ 0.1	(11) 0.3 $\pm$ 0.1	(10) 0.4 $\pm$ 0.1	(10) 0.4 $\pm$ 0.1	(10) 0.3 $\pm$ 0.1	(8) 0.4 $\pm$ 0.1	(10) 0.5 $\pm$ 0.1 ^{e,f}	
24	F2	M+F	(8) 0.5 $\pm$ 0.1	(10) 0.9 $\pm$ 0.1 ^e	(10) 1.6 $\pm$ 0.6 ^e	(10) 2.6 $\pm$ 0.6 ^e	(10) 0.6 $\pm$ 0.1	(10) 1.7 $\pm$ 0.3 ^{e,f}	(10) 2.6 $\pm$ 0.8 ^{e,f}	

^aThe 115-d-old females were full-term pregnant females; the 135-d-old females had delivered and nursed litters for 24 d.

^bMale and female values combined because of a lack of significant ($p < 0.05$) sex difference at specified age.

^cSample number.

^dMean $\pm$ SD.

^eSignificantly different from 50 (control-240 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).

^fSignificantly different from 50 (20 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).

^gWhole brain.

TABLE 5. Concentration of Manganese ($\mu\text{g/g}$ wet weight) in Selected Tissues during Chronic Mn_3O_4 Exposure

Tissue	Age	Sex ^d	Fe = 240 μg/g diet; dietary Mn (μg/g) =				Fe = 20 μg/g diet; dietary Mn (μg/g) =			
			50	400	1100	3550	50	400	1100	
Testes	24	M	0.5 ± 0.03 ^c (5) ^b (6)	0.7 ± 0.08 ^d (6)	0.8 ± 0.2 ^d (6)	1.3 ± 0.2 ^d (6)	0.7 ± 0.05 ^d (6)	0.9 ± 0.06 ^{d,e} (6)	1.2 ± 0.2 ^{d,e} (6)	
	40	M	0.6 ± 0.2 (6)	0.6 ± 0.07 (6)	0.6 ± 0.2 (6)	0.8 ± 0.2 (6)	0.6 ± 0.03 (6)	0.8 ± 0.2 (6)	0.9 ± 0.3 (6)	
	60	M	0.4 ± 0.1 (8)	0.4 ± 0.06 (7)	0.5 ± 0.06 (8)	0.5 ± 0.06 (8)	0.4 ± 0.1 (7)	0.5 ± 0.1 (8)	0.5 ± 0.04 (8)	
	224	M	0.4 ± 0.04 (5)	0.3 ± 0.05 (6)	0.4 ± 0.1 (6)	0.4 ± 0.2 (6)	0.3 ± 0.06 (6)	0.4 ± 0.06 (6)	0.4 ± 0.05 (6)	
Blood	24	M	0.02 ± 0.004 (11)	0.04 ± 0.01 ^d (12)	0.1 ± 0.05 ^d (12)	0.36 ± 0.05 ^d (12)	0.05 ± 0.01 ^d (12)	0.08 ± 0.03 ^{d,e} (12)	0.12 ± 0.06 ^{d,e} (12)	
	40	M+F	0.02 ± 0.005 (6)	0.02 ± 0.005 (10)	0.05 ± 0.02 ^d (8)	0.14 ± 0.07 ^d (9)	0.04 ± 0.01 ^d (10)	0.06 ± 0.02 ^{d,e} (8)	0.14 ± 0.07 ^{d,e} (10)	
Placenta (F ₁)	115	F	0.2 ± 0.1 (3)	0.3 ± 0.1 (2)	0.3 ± 0.04 (2)	0.4 ± 0.06 ^d (2)	0.3 ± 0.05 (3)	0.3 ± 0.04 (2)	0.4 ± 0.1 ^{d,e} (2)	
Milk (P ₀)	135	F	0.3 ± 0.1 (7)	0.2 ± 0.01 (8)	0.3 ± 0.04 (3)	0.4 ± 0.01 (7)	0.2 ± 0.1 (4)	0.2 ± 0.04 (9)	0.4 ± 0.01 ^e (7)	
Milk (F ₁)	135	F	0.2 ± 0.03 (16)	0.3 ± 0.04 ^d (14)	0.2 ± 0.04 (16)	0.4 ± 0.2 (16)	0.3 ± 0.1 (15)	0.4 ± 0.1 ^d (16)	0.4 ± 0.2 (8)	
Spleen	224	M+F	0.3 ± 0.1 (16)	0.4 ± 0.1 ^d (12)	0.3 ± 0.1 (16)	0.4 ± 0.1 (16)	0.4 ± 0.07 ^d (13)	0.4 ± 0.1 ^d (15)	0.3 ± 0.05 (8)	
Pituitary	224	M+F	0.5 ± 0.1 (16)	0.7 ± 0.1 ^d (11)	0.8 ± 0.1 ^d (12)	0.9 ± 0.1 ^d (12)	0.8 ± 0.1 ^d (10)	0.8 ± 0.1 ^d (12)	0.8 ± 0.1 ^d (8)	
Adrenals	224	M+F	2.9 ± 0.4 (16)	3.1 ± 0.4 (11)	2.7 ± 0.6 (12)	4.1 ± 0.6 ^d (12)	2.6 ± 0.2 (10)	3.2 ± 0.5 (12)	3.0 ± 0.6 (8)	

^aMale and female values combined because of a lack of significant ($p < 0.05$) sex difference at specified age.^bSample number.^cMean $\pm$ SD.^dSignificantly different from 50 (control-240 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).^eSignificantly different from 50 (20 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).

TABLE 6. Concentration of Iron ($\mu\text{g/g}$ wet weight) in Liver during Chronic Mn_2O_3 Exposure

TABLE 6. Concentration of Iron ($\mu\text{g/g}$ wet weight) in Liver during Chronic Mn_2O_3 Exposure										
Age ^d	Generation	Sex ^c	Fe = 240 $\mu\text{g/g}$ diet; dietary Mn ($\mu\text{g/g}$) =				Fe = 20 $\mu\text{g/g}$ diet ^b ; dietary Mn ($\mu\text{g/g}$) =			
			50 (control)	400	1100	3550	50	400	1100	
24	F ₁	M	(5) ^d 182 $\pm$ 65 ^e	(6) 134 $\pm$ 65	(6) 122 $\pm$ 36	(6) 36 $\pm$ 5 ^f	(6) 25 $\pm$ 2 ^f	(6) 28 $\pm$ 2 ^{f,g}	(6) 25 $\pm$ 4 ^f	
40	F ₁	M	(6) 186 $\pm$ 57	(6) 192 $\pm$ 32	(6) 120 $\pm$ 27	(6) 76 $\pm$ 20 ^f	(7) 35 $\pm$ 18 ^f	(6) 28 $\pm$ 2 ^f	(6) 29 $\pm$ 3 ^f	
40	F ₁	F	(5) 229 $\pm$ 32	(6) 234 $\pm$ 38	(6) 163 $\pm$ 48	(7) 83 $\pm$ 19 ^f	(7) 36 $\pm$ 6 ^f	(6) 29 $\pm$ 4 ^{f,g}	(6) 30 $\pm$ 4 ^{f,g}	
60	F ₁	M	(6) 160 $\pm$ 30	(6) 140 $\pm$ 34	(6) 141 $\pm$ 48	(6) 73 $\pm$ 15 ^f	(6) 83 $\pm$ 45 ^f	(6) 37 $\pm$ 4 ^{f,g}	(6) 34 $\pm$ 3 ^{f,g}	
60	F ₁	F	(5) 160 $\pm$ 40	(6) 237 $\pm$ 61	(6) 197 $\pm$ 43	(6) 127 $\pm$ 28	(6) 55 $\pm$ 25 ^f	(6) 36 $\pm$ 8 ^f	(5) 24 $\pm$ 2 ^{f,g}	
100	F ₁	M	(6) 216 $\pm$ 80	(6) 131 $\pm$ 18	(6) 174 $\pm$ 73	(6) 135 $\pm$ 66	(6) 52 $\pm$ 10 ^f	(6) 44 $\pm$ 10 ^f	(6) 45 $\pm$ 10	
100	F ₁	F	(6) 282 $\pm$ 108	(6) 252 $\pm$ 51	(6) 221 $\pm$ 52	(5) 193 $\pm$ 11	(6) 78 $\pm$ 15 ^f	(6) 56 $\pm$ 8 ^{f,g}	(6) 47 $\pm$ 5 ^{f,g}	
115	F ₁	F	(7) 192 $\pm$ 54	(10) 198 $\pm$ 63	(9) 177 $\pm$ 44	(9) 113 $\pm$ 20 ^f	(10) 54 $\pm$ 13 ^f	(8) 46 $\pm$ 10 ^f	(10) 33 $\pm$ 7 ^{f,g}	
135	F ₁	F	(8) 184 $\pm$ 39	(9) 185 $\pm$ 32	(5) 171 $\pm$ 50	(5) 119 $\pm$ 43 ^f	(5) 51 $\pm$ 11 ^f	(10) 43 $\pm$ 12 ^f	(8) 43 $\pm$ 6 ^f	
			(8)	(7)	(8)	(8)	(7)	(8)	(8)	

224	F ₁	M	182 ± 36	198 ± 30	174 ± 29	118 ± 27 ^f	123 ± 32 ^f	99 ± 22 ^f	72 ± 15 ^{f,g}
			(5)	(8)	(8)	(8)	(8)	(8)	
224	F ₁	F	326 ± 128	349 ± 100	403 ± 80	261 ± 51	128 ± 28 ^f	115 ± 23 ^f	(h)
			(7)	(10)	(10)	(10)	(10)	(8)	(10)
Term pups	F ₂	M+F	264 ± 36	276 ± 75	261 ± 57	286 ± 52	146 ± 33 ^f	95 ± 32 ^{f,g}	104 ± 44 ^{f,g}
			(8)	(10)	(10)	(10)	(10)	(10)	(10)
24	F ₂	M+F	195 ± 48	154 ± 63	97 ± 42 ^f	54 ± 25 ^f	46 ± 61 ^f	21 ± 2 ^f	23 ± 2 ^f

^aThe 115-d-old females were full-term pregnant females; the 135-d-old females had delivered and nursed litters for 24 d.

^bLiver Fe concentrations in the low dietary Fe (20 ppm Fe) group are significantly ($p < 0.01$) lower than in the normal dietary Fe (240 ppm Fe) group.

^cMale and female values combined because of a lack of significant ($p < 0.05$) sex difference at specified age as indicated.

^dSample number.

^eMean ± SD.

^fSignificantly different from 50 (control-240 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).

^gSignificantly different from 50 (20 ppm Fe) group by ANOVA and Dunnett's multiple comparison test ($p < 0.05$).

^hInsufficient F₁ offspring to complete this group.

the Fe-sufficient diet supplemented with Mn_3O_4 showed no significant Mn-related depressions in Fe concentration. All animals on the Fe-deficient diet had significantly lower tissue Fe concentrations than those on the Fe-sufficient diet. In general, the addition of substantial amounts of Mn to the low-Fe diet further depressed Fe concentrations in these tissues.

Fe concentrations were determined in spleen from 224-d-old animals. The Fe depletion pattern in the spleen of animals on the Fe-sufficient diet supplemented with Mn_3O_4 was similar to that in the liver. However, Fe deficiency without Mn supplement produced spleen Fe concentrations that were 40% of those of the normal Fe controls. Concurrent Mn exposure (1100 $\mu g/g$ diet) produced spleen Fe concentrations that were only 10% of the control value (240 $\mu g/g$ Fe).

DISCUSSION

In general, chronic Mn_3O_4 exposure resulted in increased tissue accumulation of Mn. Dietary Fe deficiency in addition to chronic Mn_3O_4 exposure resulted in additional increases in tissue Mn.

The Fe-deficient animal is apparently more sensitive to chronic Mn_3O_4 exposure, as evidenced by enhanced tissue accumulation of Mn, growth retardation, and a high incidence of mortality in animals receiving 3550 $\mu g/g$ Mn in a low-Fe diet.

Prewanling rats had a Mn tissue distribution of liver > kidney = cerebrum > testes at 24 d of age. However, preweanling rats gavaged daily with Mn_3O_4 from birth had a Mn tissue distribution of cerebrum = liver > kidney > testes at 24 d of age (Rehnberg et al., 1980). Compared to animals in this study receiving dietary Mn_3O_4 , the gavaged animals had cerebral Mn concentrations that were four times higher and kidney concentrations of Mn that were approximately doubled. Animals on the normal feeding pattern received little Mn in the dam's milk prior to 15 d of age, when consumption of a laboratory diet containing substantial amounts of Mn_3O_4 began. Gavaged animals received considerable amounts of Mn_3O_4 daily from birth, and Mn absorption and retention are highly influenced by age. The younger the animals at exposure, up to approximately 15 d postpartum, the higher the retention of Mn (Cahill et al., 1980). It has also been suggested that the capacity of the infant's liver to sequester Mn can be exceeded, with the excesses being released to other organs (Cahill et al., 1980).

Liver Fe concentrations appeared to be related to Mn exposure only in the F_1 and F_2 24-d-old weanlings and in term and lactating dams. In general, chronic Mn exposure of rats on either dietary regimen showed only minor depressions in tissue Fe concentrations, with the exception of liver and spleen.

Irrespective of dietary Fe level, liver Mn concentrations were not affected during gestation, while liver Fe concentrations were depressed 30–40% at term (115 d of age). During lactation, liver Mn concentrations were increased

30-50% and liver Fe concentrations were not additionally depressed. This dramatic increase in liver Mn may be due to the increased food intake during lactation. Pietrzak-Flis et al. (1978) reported a 130% increase in food consumption during lactation compared to that of the nonlactating animal.

Other studies (Rehnberg et al., 1980, 1981) have also shown that chronic Mn exposure of the young rat results in high brain and pituitary Mn concentrations. Occupational exposure to Mn dust can result in disorders of the central nervous system with symptoms similar to those of Parkinson's disease. Brain damage appears to be caused by flooding with Mn; however, symptoms persist after flooding ceases, suggesting structural injury (Mena, 1974). There is also direct evidence that chronic manganism and parkinsonism share similar biochemical abnormalities, particularly a decrease in brain dopamine (Cotzias et al., 1976). Other studies have shown post-pubertal Mn alterations in locomotor activity of rodents chronically exposed to Mn_3O_4 (Gray and Laskey, 1980; Gray et al., 1978; J. W. Laskey, in preparation). Rodier (1955), in studies of Mn miners with symptoms of Mn toxicity, observed alterations in their excretion of androgen metabolites.

To summarize the information obtained in this study, the following points are noted. Chronic dietary Mn_3O_4 results in dose-related increases in tissue Mn accumulation, and concomitant Fe deficiency promotes Mn accumulation. With chronic particulate Mn_3O_4 exposure, tissue Mn concentrations are highest in the weanling, while rats 40 d old or older, which are protected by biliary excretion and tissue barriers, accumulate minimal amounts of Mn. Although maximal dietary exposure (milligrams per kilograms of body weight per day) occurs between 24 and 40 d of age, all tissues exhibited a decline in Mn concentration during this period. This indicates that Mn uptake and retention are maximal during the preweanling period. Chronic particulate Mn_3O_4 exposure of rats on a normal Fe diet results in significant Mn absorption at Mn levels of 3550 $\mu\text{g/g}$ diet, as indicated by liver concentrations in animals through 100 d of age. Animals receiving 3550 $\mu\text{g/g}$ Mn in a normal Fe diet had elevated tissue concentrations of Mn through 224 d of age. Animals on the low-Fe diet supplemented with 400 and 1100 $\mu\text{g/g}$ Mn had significantly elevated liver Mn concentrations through 224 d of age.

These results indicate that the populations most susceptible to increased Mn accumulation are those absorbing large amounts of Fe, the preweanling, the lactating dam, and the Fe-deficient rat.

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